AMENDMENTS TO THE SPECIFICATION:

Please add the following new paragraphs after paragraph [0011]:

Brief Description of the Drawings

Fig. 1 shows the results of one dimensional SDS-PAGE electrophoresis.

Fig. 2A shows the results of two-dimensional electrophoresis in the absence of Activation Buffer.

Fig. 2B shows the results of two-dimensional electrophoresis in the presence of Activation Buffer.

Fig. 2C shows the results of two-dimensional electrophoresis using DHFR instead of CaMKIIδ.

Fig. 3 shows analysis charts of separation by MALDI-TOFMS of phosphorylated spots after trypsin treatment. From the peptide patterns, they were identified to be eIF4B and STIP1 respectively.

Fig. 4 shows a figure by in vitro assay. It was observed that GST-fused eIF4B and STIP1 were markedly phosphorylated by CaMKIIδ.

Fig. 5 shows a figure by semi-intact phosphorylation assay. The phosphorylation of eIF4B and STIP1 were observed in CaMKIIδ gene-transfected HeLa cell.

Please replace paragraph [0030] with the following amended paragraph:

[0030]

As commercially available cell-free protein synthesis system, the ones derived from E. Coli such as E. Coli S30 ~~extract system~~ EXTRACT SYSTEM (supplied by Promega) and RTS 500 ~~Rapid Translation System~~ RAPID TRANSLATION SYSTEM (supplied by Roche), and the ones derived from rabbit reticulocytes such as ~~Rabbit Reticulocyte Lysate System~~ RABBIT RETICULOCYTE LYSATE SYSTEM (supplied by Promega) may be mentioned.

However, for a cell-free protein synthesis system used in the present invention, gramineous plants such as wheat, barley, rice and corn are preferred. A cell-free protein synthesis system derived from a wheat embryo is particularly preferred.

Please replace paragraph [0037] with the following amended paragraph:

[0037]

As a dialysis membrane for use in the removing operation of low molecular synthesis inhibitors through dialysis, the one which can remove a molecule having a molecular weight of 50,000 to 12,000 may be mentioned, specifically, a recyclable cellulose membrane (from Viskase Sales, Chicago) which can remove a molecule having a molecular weight of 12,000 to 14,000, ~~Spectra/Pore 6~~ SPECTRA/PORE 6 (from SPECTRUM LABOTRATORIES INC., CA, USA) which can remove a molecule having a molecular weight of 50,000, and the like may preferably be used. A suitable amount of the wheat embryo extract-containing solution will be

put toward the one-side of such dialysis membrane, and then dialysis is conducted by a conventional method. The dialysis is preferred to be conducted for 30 minutes to 24 hours.

Please replace paragraph [0045] with the following amended paragraph:

[0045]

For specific methods for decreasing a reducing agent, there is used a method wherein a wheat embryo extract-containing solution is prepared to be free from a reducing agent, and then supplied with a reducing agent to have an above described concentration range together with necessary components for a cell-free protein synthesis system, or a method wherein a reducing agent is removed from a solution for translation reaction derived from a wheat embryo extract-containing solution to be within the concentration range described above. As a wheat embryo extract-containing solution for cell-free protein synthesis requires a high degree of reduction condition to extract, a method wherein a reducing agent is removed from the solution after extraction is easier to execute. As a method for removing reducing agent from a wheat embryo extract-containing solution, there is mentioned a method using a gelfiltration support. Specifically, for example, there is mentioned a method wherein ~~Sephadex~~ SEPHADEX G-25 column is beforehand equilibrated with an appropriate buffer containing no reducing agent, and then fed with a wheat embryo extract-containing solution to pass

through.

Please replace paragraph [0050] with the following amended paragraphs:.

[0050]

Cell-Free Protein Synthesis

(1) Preparation of Wheat Embryo Extract Solution

The seeds of Chihoku wheat produced in Hokkaido or those of Chikugoizumi produced in Ehime were fed into a mill (from Fritsch: ~~Rotor Speed Millpulverisette Type 14~~ ROTOR SPEED MILLPULVERISETTE TYPE 14) at the rate of 100g/min, and pulverized gently at a speed of 8,000 rpm. After collecting a fraction containing a germinable wheat embryo by a sieve (sieve opening from 0.7 to 1.00 mm), selection by flotation with the mixture of carbon tetrachloride and cyclohexane (volume ratio; carbon tetrachloride: cyclohexane = 2.4:1) was conducted to recover a floating fraction containing a germinable wheat embryo, then organic solvent medium was dried off at room temperature, and then mixed impurities such as seed coats were removed by blowing at room temperature to obtain a crude wheat embryo fraction.

Next, using a belt type color sorter, BLM-300K (manufacturer: Anzai Manufacturing Co., Ltd., distributor: Anzai Corporation, Ltd.), a wheat embryo was sorted out from a crude wheat embryo fraction by taking advantage of differences in color as below. This color sorter is an apparatus that comprises a means for irradiating light to the crude wheat

embryo fraction, a means for detecting reflected and/or transmitted beams from crude wheat embryo fraction, a means for comparing the detected value with a reference value, and a means for sorting them into classes without and within the scope of the reference value. A crude wheat embryo fraction was fed onto the beige-colored belt of the color sorter at a rate of 1000 to 5000 grains/cm^2, then the crude wheat embryo fraction on the belt was irradiated with the light of a fluorescent lamp and its reflected light detected. The belt was set to convey at a speed of 50m/min. As a photosensor, a monochrome CCD line sensor (2048 pixels) was used.

First, to eliminate darker color components (seed coat etc.) than a wheat embryo, a reference value was set between the wheat embryo luminance and the seed coat luminance, and the component exceeding the reference value was sucked to eliminate. Then, to sort out an endosperm, a reference value was set between the wheat embryo luminance and the endosperm luminance, and the component exceeding the reference value was sucked to eliminate. 30 suction nozzles (1 suction nozzle per 1cm length) were placed at the position approximately 1cm above the conveyer belt to suck. This method was repeated to sort the wheat embryo until it has a wheat embryo purity (a weight ratio of wheat embryo contained per 1g of random sample) of 98% or higher.

The obtained wheat embryo fraction was suspended in distilled water at 4°C, and washed with a rinsing solution in an ultrasonic cleaner until the solution got free from white

turbidity. Then, it was suspended in 0.5 v% solution of ~~Nonidet~~ NONIDET (from Nacalai Techtonics) P40, and washed with a rinsing solution in the ultrasonic cleaner until the solution got free from white turbidity to obtain wheat embryo, and then the following operations were conducted at 4°C.

An extractant (80 mM of HEPES-KOH, pH 7.8, 200 mM of potassium acetate, 10 mM of magnesium acetate, 8 mM of dithiothreitol, (each 0.6 mM of 20 kinds of L-amino acids may have been added)) of twice the volume of wet weight of the washed wheat embryo was added, and then the wheat embryo was limitedly homogenized 3 times at 5,000 to 20,000 rpm for 30 seconds using a ~~Waring~~ WARING blender. This homogenate was centrifuged at 30,000 x g for 30 minutes using a high-speed centrifuge to give a supernatant, which was centrifuged again in a similar condition to obtain a supernatant. This sample was subjected to long-term storage at -80°C or below, resulting in no deterioration of the activity. The obtained supernatant was filtered with a filter having a pore size of 0.2μm (NEW ~~Steradisc 25~~ STERADISC 25: supplied by Kurabo Industries Ltd.) to sterilize by filtration and eliminate contaminating fine dusts.

Next, this filtrate was subjected to gelfiltration using ~~Sephadex G-25~~ SEPHADEX G-25 column which had been equilibrated with a solution [40 mM of HEPES-KOH (pH 7.8), and the mixture of, respectively, 100 mM of potassium acetate, 5 mM of magnesium acetate, 8 mM of dithiothreitol, each 0.3 mM of 20 kinds of L-

amino acids (amino acids may be present, absent or labeled depending on the purpose of protein synthesis)] in advance. The obtained filtrate was centrifuged again at 30,000 x g for 30 minutes to recover a supernatant, which was adjusted to have a concentration of 90 to 150 at A260nm (A260/A280=1.4 to 1.6). To the obtained wheat embryo extract-containing solution for protein synthesis, 20 mM of HEPES-KOH (pH 7.6), 100 mM of potassium acetate, 2.65 mM of magnesium acetate, 0.380 mM of spermidine (from Nacalai Techtonics), each 0.3 mM of 20 kinds of L-amino acids, 4 mM of dithiothreitol, 1.2 mM of ATP (from Wako Pure Chemical Industries, Ltd.), 0.25 mM of GTP (from Wako Pure Chemical Industries, Ltd.), 16 mM of phosphocreatine (from Wako Pure Chemical Industries, Ltd.), 1000U/ml of Rnaseinhibitor (from TAKARA), 400μg/ml of creatine kinase (from Roche) were added to prepare the source of solution for translation reaction.

Please replace paragraph [0053] with the following amended paragraph:

[0053]

(1) Assay for HeLa Cell-Extract Solution Using CaMKIIδ as Trigger

Preparation of HeLa Cell-Extract Solution

HeLa cell was cultured to confluency in a 10 cm culture dish by a conventional method. The cells were collected with a cell scraper, placed in a 50mL centrifuge tube containing 20mL of PBS (-) [137 mM NaCl, 8.1 mM Na_2HPO_4, 2.68 mM KCl, 1.47 mM

KH_2PO_4], centrifuged (3,000 rpm, 2 min, 4°C) to discard the supernatant, and suspended /centrifuged in 20 mL of fresh supplied PBS (-) three times to wash. The obtained cell mass was suspended again in 20 mL of PBS (-), separated into 1.5 mL tubes by every 1 mL, centrifuged (15,000 rpm, 3 min, 4°C) to discard the supernatant and stored at -80°C. For reaction, usually, the stored 10 tubes were used to give one unit, melt, centrifuged (15,000 rpm, 5 min, 4°C) to discard the supernatant gently, suspended again in 10 µL of a cell extract buffer [50 mM Tris-HCl (pH7.5), 1 mM EDTA, 6 mM β-mercaptoethanol], subjected to repletion of freezing in liquid nitrogen and melting at room temperature to burst cells, united to give one sample, which was then subjected to exchange of buffer and removal of intrinsic low molecular compounds using ~~Sephadex G-25~~ SEPHADEX G-25 column equilibrated with 1 x Reaction Buffer [50 mM Tris hydrochloride (pH7.6), 10 mM magnesium hydrochloride, 0.5 mM dithiothreitol], and centrifuged at 15,000rpm for 1 minute to obtain the supernatant as a cell extract solution.

Please replace paragraph [0060] with the following amended paragraph:

[0060]

(3) In Vitro Phosphorylation Assay

Reaction with CaMKIIδ was confirmed using a partially purified protein substrate which used a glutathione column. ~~Glutathione-Sepharose4B~~ GLUTATHIONE-SEPHAROSE 4B (Amersham)

resin was washed with 10 times amount of 1 x Reaction buffer three times, and a ~~Glutathione-Sepharose 4B~~ GLUTATHIONE-SEPHAROSE 4B resin suspension was prepared in 1 x Reaction buffer which had an equal volume to the column size. 20 μL of the above described resin suspension was prepared for each sample, supplied with 20μL of reaction solutions in which GST fused protein substrates (GST-elF4B, GST-STIP1) were synthesized, and incubated (4°C, 1 h) to bind the GST fused protein substrate to the above described resin. The resin was centrifuged(800 x g, 5 min) to discard the supernatant, washed once again with 200μL of 1 x Reaction buffer, centrifuged again to discard the supernatant in the same manner as above, supplied with 10 μL of CaMKIIδ exchanged in 1 x Reaction buffer using ~~SephadexG-25~~ SEPHADEX G-25 column, 3 μL of 5X Activation buffer and 2 μL of 5 fold-diluted [γ-^{32}P] ATP, incubated (30°C, 20 min), centrifuged (800 x g, 5 min) to discard the supernatant, washed three times with 200 μL of 1 x Reaction buffer, supplied with 15 μL of 1X SDS-Sample buffer, and boiled for 5 minutes. 7.5 μL of the resultant was migrated (12.5 % e-PAGEL, Atto Corporation) to give a gel, which was dried and subjected to autoradiography (BAS-2500, FUJI PHOTO FILM CO., LTD.) to analyze the images (Fig. 4) obtained by radiation. As a result, there was reproduced the reaction in which GST-fused eIF4B and STIP1 were phosphorylated by CaMKIIδ. Thereby, the in vitro assay also confirmed that the spots separated and identified through two-dimensional electrophoresis were eIF4B and STIP1.

Please replace paragraph [0061] with the following amended paragraph:

[0061]

(4) Semi-Intact Phosphorylation Assay

CaMKIIδ was integrated into transfection vector pcDNA3.1 (-) (Invitrogen) and the gene was introduced into HeLa cell according to a conventional method. The cells collected were supplied with 50 μL of extraction buffer and subjected to five repetitions of freeze-thawing to burst the cells. The obtained HeLa cell extract solution was exchanged in 1 x Reaction buffer using ~~Sephadex G-25~~ SEPHADEX G-25 column. Then, 30 μL of buffer exchanged HeLa cell extract solution was supplied with 4.5 μL of 1 mM ATP, 3 μL of 3 x Reaction buffer and 9 μL of 3 x Activation buffer, preincubated (30°C, 20 min), supplied with 3 μL of [γ-^{32}P]ATP and incubated (30°C, 20 min) to conduct phosphorylation reaction. After reaction, the whole volume was spread out by two-dimensional electrophoresis to give a gel, which was then dried and subjected to autoradiography (BAS-2500, FUJI PHOTO FILM CO., LTD.) to confirm phosphorylated spots (Fig. 5). As a result, it was demonstrated that eIF4B and STIP1 were also phosphorylated in CaMKIIδ-transfected cells. As CaMKIIδ was confirmed to express in HeLa cell, eIF4B and STIP1 are thought to be the natural substrates of CaMKIIδ.

Please delete paragraph 0063 as follows:

~~Brief Description of the Drawings~~

~~[0063]~~

~~Fig. 1 shows the results of one dimensional SDS-PAGE electrophoresis.~~

~~Fig. 2A shows the results of two-dimensional electrophoresis in the absence of Activation Buffer.~~

~~Fig. 2B shows the results of two-dimensional electrophoresis in the presence of Activation Buffer.~~

~~Fig. 2C shows the results of two-dimensional electrophoresis using DHFR instead of CaMKIIδ.~~

~~Fig. 3 shows analysis charts of separation by MALDI-TOFMS of phosphorylated spots after trypsin treatment. From the peptide patterns, they were identified to be eIF4B and STIP1 respectively.~~

~~Fig. 4 shows a figure by in vitro assay. It was observed that GST-fused eIF4B and STIP1 were markedly phosphorylated by CaMKIIδ.~~

~~Fig. 5 shows a figure by semi-intact phosphorylation assay. The phosphorylation of eIF4B and STIP1 were observed in CaMKIIδ gene-transfected HeLa cell.~~